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Short courses of dual-strain probiotics appear to be effective in reducing necrotising enterocolitis

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Abstract

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Methods: Retrospective cohort study of neonates (birth weight 400-1,500 g) in three neonatal intensive care units in Switzerland and Germany that embarked on probiotic prophylaxis given for 10 or 14 days, employing a fixed combination (*Lactobacillus acidophilus* plus *Bifidobacterium infantis*, each 10^9 CFU/d) licensed as a drug in Switzerland. Probiotics were initiated upon discontinuation of antibiotics, or on day 1-3 in infants without antibiotics. Repeat probiotic courses were given whenever antibiotics had been instituted and were discontinued.

Results: Birth weight and gestational age were similar in the two 24-month pre- and post-implementation cohorts. NEC rates fell from 33/633 (5.2%) to 8/591 infants alive at 3 days (1.4%; risk ratio (RR) 0.26, 95% confidence interval (CI) 0.12-0.55). The drop in NEC was significant both for infants of 400-999 g (6.4% to 2.5%) and 1,000-1,500 g birth weight (4.4% to 0.6%). Mortality was 5.1% (32/633) without, as opposed to 3.5% (21/591) with probiotics, respectively (RR 0.69, 95% CI 0.41-1.19).

Conclusion: Short courses of a dual-strain probiotics appear to be effective in reducing NEC.

Key notes

- The risk of necrotising enterocolitis decreases with the use of prophylactic probiotics but the optimal duration is unknown.
- In most trials, prophylactic probiotics were given over prolonged periods of time, sometimes until discharge.
- In our study, dual-strain probiotics consisting of *Lactobacillus acidophilus* and *Bifidobacterium infantis* were highly effective in reducing rates of necrotising enterocolitis when given for 10-14 days, with repeat courses after any institution of antibiotics.

Introduction

Initial colonisation of the neonatal intestinal tract has short and long-term consequences, as some bacteria play important roles in regulating mucosal immunity and establishing barrier formation, whereas others may cause inflammatory disease. Alterations of gut bacterial colonisation observed in preterm, as compared to term infants, are aggravated by exposure to antibiotics before and after delivery. Antibiotics have been shown to affect the composition of intestinal microbiota for up to 90 days, decreasing abundance of bifidobacteria while increasing enterobacteriaceae (1). Such shifts in microbial composition may turn into intestinal dysbiosis that contributes to the development of necrotising enterocolitis (NEC) (2-5). Both prenatal (6, 7) and postnatal antibiotics (8-10) have been found to be associated with NEC in very low birth weight (< 1,500 g, VLBW) infants.

Probiotics can be viewed as an attempt to correct for the side effects of perinatal antibiotics in preterm infants and appear to counteract their NEC promoting effects. Meta-analyses of randomised controlled trials suggest that prophylactic administration of probiotics reduces the rates of NEC and all-cause mortality in VLBW infants (11), and introduction of routine administration of probiotics is associated with a decline in the

incidence of NEC (12-16). Various clinicians and investigators, however, have voiced concerns, as probiotic bacteria used as NEC prophylaxis have been isolated in blood cultures of sick very low birth weight infants (17-19). Moreover, the most effective probiotic or combination of probiotics, dosage and timing are unknown (20). A comparison of the results of randomised controlled trials using probiotics containing combinations of two or more bacterial species with trials employing single-strain probiotics suggests that combination probiotics are more effective than single-strain probiotics in reducing rates of NEC and mortality (11). The optimal duration of probiotics administration remains an open question.

After the results of the first two single randomised controlled trials that demonstrated a significant reduction of NEC and mortality in VLBW infants with the use of prophylactic multiple-strain probiotics became known (21, 22), three tertiary care neonatal intensive care units of the university hospitals of Zürich (Switzerland) and Berlin (Germany) sequentially embarked on an institutional policy to prescribe probiotics combining *Lactobacillus acidophilus* and *Bifidobacterium infantis* licensed by the Federal Swiss Agency for Therapeutic Products (Swissmedic) for use in infants (Infloran). In contrast to schemes employed elsewhere, the institutional guidelines called for the probiotics to be administered only for 10 or 14 days, with repeat courses after a second or third round of antibiotics for suspected or proven nosocomial infection. The short exposure time, as compared to those of the protocols of the randomised controlled trials, was chosen as intestinal colonisation with probiotic strains appears to persist at least two weeks after cessation of probiotic administration (23). There were also concerns about the concomitant administration of probiotics and broad-spectrum antibiotics (24). Here, we report NEC and mortality rates before and after introduction of this abbreviated scheme of prophylactic dual-strain probiotics administration.

Patients and methods

The routine use of probiotics for VLBW infants was started in September 2007 (University Hospital Zürich), in July 2008 (Charité Berlin, Campus East) and in November 2008 (Charité Berlin, Campus West). VLBW infants received ½ capsule of Infloran (Laboratorio Farmaceutico, Mede, Italy) containing *Lactobacillus acidophilus* ($1x10^9$ CFU) and *Bifidobacterium infantis* ($1x10^9$ CFU) twice daily (total daily dose $1x10^9$ CFU of each organism) for 10 (Berlin) or 14 (Zürich) consecutive days. Probiotics were started on day 1 (Zürich) to 3 (Berlin) of life in infants who had not been put on antibiotics at admission. In infants given antibiotics upon admission, probiotic prophylaxis was started on the very day antibiotics were discontinued. Probiotic prophylaxis was repeated (again for 10 or 14 days, respectively) after further rounds of antibiotics as long as infants had an actual body weight below 1,500 g or an actual corrected gestational age below 32 weeks.

The feeding regimen of the three units similarly called for maternal milk started within the first 24 hours of life. If maternal milk was unavailable, infants received donor milk in Berlin unless the mother objected, while they received preterm formula in Zürich.

In this analysis, we used the anonymised quality control data sets (25) to compare rates of mortality and NEC stage 2 or more in VLBW infants who had survived for at least 72 hours between two 24-month periods (before and 6 months after introduction of probiotic prophylaxis, respectively). The study was approved by the two local institutional review boards (KEK-ZH-Nr 2014-0512, EA2/130/14).

Groups were compared by the Mann Whitney U, chi square test or two-tailed Fisher's exact test, using Statgraphics Plus 5.0; (Statistical Graphics Corp., Warrenton, Virginia, USA). Risk ratios (RR) with 95% confidence intervals (CI) (Mantel and Haenszel) and heterogeneity (Higgins) were calculated using the software Review Manager (RevMan 5.1.2; The Cochrane Collaboration, London, England). Fixed analyses were calculated after heterogeneity between the three NICUs was found to be low.

Results

In the two 24-month periods before and after routine use of probiotic prophylaxis, the three tertiary care NICUs admitted 700 and 652 infants, of whom 67 and 61 infants, respectively, died within the first 72 hours of life (p=0.926). Patients' characteristics of infants alive at 72 hours and therefore included in the analysis were similar for the two periods (Table 1). The incidence of NEC (≥ stage 2) decreased from 5.2% (33/633) to 1.4% (8/591), translating into a risk RR of 0.26 (95% CI 0.12-0.55). The reduction in NEC was significant for the strata of infants with a birth weight of 400-999 g and 1,000-1,500 g, being somewhat more pronounced in infants with a birth weight ≥ 1,000 g (Table 2). Rates of mortality did not differ significantly between the two time periods (5.1% vs 3.6%). There was no change in survivors with respect to the rates of retinopathy of prematurity ≥ stage 2 (10.3%, 62/601, vs 12.3%, 70/570) or chronic lung disease, as defined by oxygen requirement at 36 weeks gestational age (9.8%, 59/601, vs 8.9%, 51/570).

Discussion

This retrospective cohort study suggests that 10 or 14-day courses of probiotics started immediately after elective cessation of antibiotics are as effective in reducing the rates of NEC in preterm infants with a birth weight < 1,500 g as continuous administration of probiotics for prolonged periods of time. The RR of NEC for infants < 1,500 g birth weight associated with the use of a short course of probiotics (0.26, 95% CI 0.12-0.55) was similar to that of the most recent meta-analysis (11) of all published randomised controlled trials (0.41, 95% CI 0.31-0.56) or randomised controlled trials using multiple-strain probiotics (0.37, 95% CI 0.25-0.54). The comparison of the pre-implementation and post-implementation cohorts failed to demonstrate a significant reduction in mortality.

The reduced rates of NEC associated with the introduction of probiotics might have been linked to other changes in practice, a problem inherent to similar longitudinal studies (12-16). Other, yet unaccounted factors may have contributed to the reduced NEC rates observed, such as less antenatal antibiotics, more breastfeeding, or emphasis on late cord clamping. These practice parameters are not contained in the anonymised quality control data sets the analysis is based upon. The effect size, however, was similar for the units in Zürich and Berlin, despite the difference in duration of probiotics (14 days versus 10 days) and disparities in feeding regimens for infants whose mothers did not provide breast milk (preterm formula in Zürich, donor bank milk in Berlin). In both places, probiotics tended to influence NEC-related mortality (RR 0.27, 95% CI 0.06-1.26) but not mortality unrelated to NEC (RR 0.93, 95% CI 0.55-1.58). There was little variation in case mix between the pre- and post-implementation cohorts, as judged by birth weight and gestational age.

Probiotics consist of live bacteria differing in their efficacy to colonise various segments of the gut (26). It may be hypothesised that they multiply in the intestinal lumen unless killed by antibiotics (23), rendering it pointless to provide additional probiotic bacteria once colonisation is firmly established. The results of our analysis suggest that a short course (10-14 days) of probiotics consisting of *Lactobacillus acidophilus* and *Bifidobacterium infantis* may be sufficient to prevent NEC, provided further courses of probiotics are given to infants put on intravenous antibiotics for nosocomial infections. While the data set used for the analysis did not directly provide numbers to show how many infants had more than one course of probiotics, data from the neonatal nosocomial surveillance system (27) indicated that 20% of VLBW infants of the Berlin cohort 2009-2011 had any type of nosocomial infection beyond 3 days of life (including culture-negative suspected clinical sepsis), and 0.8% had two such nosocomial infections. In the Zürich cohort, 7.7% of VLBW infants had one culture-positive nosocomial septicaemia, and 0.3% had two culture-positive septicaemias. This translates into about 19.3 % and 0.8% of VLBW infants, assuming that culture-positive septicaemias account for 40% of all nosocomial infections (27).

Critics of trials employing probiotics to prevent NEC voiced their concerns that trials were conducted using probiotics marketed as dietary supplements that are not produced under stringent quality control and lack regulatory approval by national drug regulatory authorities. The probiotics employed in our units is a licensed drug in Switzerland for the use in infants (aimed at shortening the duration of diarrhoea). Its prescription to individual patients is covered by legislation in most European countries. While these probiotics were found to reduce rates of NEC and mortality in two large randomised controlled trials conducted in Taiwan (21, 28), it has been questioned whether these results would also apply to a central European setting (20). The data presented here reflect results of large neonatal intensive care units featuring NEC and mortality figures representative of Germany and Switzerland (25, 27).

Conclusion

The results of our study suggest that 10-14 day courses of dual-strain probiotics containing Lactobacilli and Bifidusbacteria, with repeat courses of probiotics after antibiotic administration, may be as efficient in reducing the rates of NEC as more prolonged administration of probiotics.

Competing Interest: None declared.

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Table 1: Demographic data of the VLBW (400-1500 g) infants born in the three NICUs before and after introducing Infloran for routine use.

NICU:	**Zürich**		**Berlin East**		**Berlin West**		**all**	
Probiotics:	No	Yes	No	Yes	No	Yes	No	Yes
Infants alive at 72 h (n)	220	217	151	156	262	218	633	591
Sex male (n, %)	101 (46)	111 (51)	81 (54)	76 (49)	118 (45)	111 (51)	302 (48)	298 (50)
Birth weight (g)								
Mean	1116	1123	1054	1065	1069	1043	1082	1078
Median	1165	1150	1090	1075	1075	1070	1110	1100
Range	560-1500	530-1500	400-1490	480-1490	407-1495	439-1495	400-1500	439-1500
IQR	900-1330	880-1350	850-1280	840-1330	860-1320	777-1300	880-1320	850-1330
< 500 g (n, %)	0 (0)	0 (0)	4 (2.7)	2 (1.3)	5 (1.9)	5 (2.3)	9 (1.4)	7 (1.2)

Gestational age (weeks)								
Mean	29.7	29.6	28.9	28.9	28.8	28.8	29.1	29.1
Median	29.4	29.6	28.7	28.4	28.4	29.0	28.9	29.0
Range	24.6-36.3	24.9-37.1	23.3-34.6	23.3-37.1	22.9-40.0	23.5-37.5	22.9-40.0	23.3-37.5
IQR	27.6-31.6	27.9-31.3	27.0-30.9	27.1-30.3	26.4-30.9	26.5-30.9	27.1-31.0	27.1-31.1

IQR – Interquartile range

Table 2: Effect of routine use of probiotics on NEC and mortality in all VLBW infants and in VLBW infants stratified by birth weight

	No probiotics	Probiotics	RR (95% CI)	p
Birth weight 400-1500 g				
Infants (n)	633	591		
Birth weight (g)	1110 (880-1320)	1100 (850-1330)		> 0.1
Gestational age (weeks)	28.9 (27.1-31.0)	29 (27.1-31.1)		> 0.1
NEC (n, %)	33 (5.2)	8 (1.4)	0.26 (0.12-0.55)	0.0005
Mortality (n, %)	32 (5.1)	21 (3.6)	0.69 (0.41-1.19)	>0.1
NEC mortality (n, %)	8 (1.3)	2 (0.3)	0.27 (0.06-1.26)	0.07
Birth weight 400-999 g				
Infants (n)	250	238		
Birth weight (g)	810 (660-930)	790 (680-885)		> 0.1
Gestational age (weeks)	26.7 (25.3-28.3)	26.6 (25.4-28.1)		> 0.1
NEC (n, %)	16 (6.4)	6 (2.5)	0.39 (0.16-0.99)	0.039

Mortality (n, %)	26 (10.4)	16 (6.7)	0.65 (0.36-1.17)	> 0.1
NEC mortality (n, %)	7 (2.8)	2 (0.84)	0.30 (0.06-1.43)	> 0.1
Birth weight 1000-1500 g				
Infants (n)	383	353		
Birth weight (g)	1270 (1150-1400)	1300 (1156-1410)		> 0.1
Gestational age (weeks)	30.3 (28.7-31.7)	30.4 (29.0-32.0)		> 0.1
NEC (n, %)	17 (4.4)	2 (0.57)	0.13 (0.03-0.55)	0.001
Mortality (n, %)	6 (1.6)	5 (1.4)	0.90 (0.28-2.94)	> 0.1
NEC mortality (n, %)	1 (0.3)	0 (0)	not estimable	> 0.1

Data for birth weight and gestational age are given as median and interquartile range